Early pregnancy bisphenol A exposure, newborn methylation and behavioural problems in childhood

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Tiivistelmä – Referat – Abstract

Tavoitteet: Bisfenoli A (BPA) on yleisesti käytetty muovin pehmitin, jolla on hormonitoimintaa häiritseviä ominaisuuksia. Sikiöaikainen altistuminen BPA:lle on yhdistetty lapsuusiän käytösoireiluun. On mahdollista, että nämä yhteydet välittyvät DNA:n metylaation muutosten kautta. Tässä tutkimuksessa tarkasteltiin, onko raskaudenaikainen BPA altistus yhteydessä lapsuusiän käytösoireiluun ja onko DNA:n metylaatioon perustuva raskaudenaikaisen BPA altistuksen biomarkkeripistemäärä yhteydessä lapsuusiän käytösoireiluun.

Menetelmät: Tutkimukseen osallistui 442 äiti-lapsi paria suomalaisesta PREDO-kohortista. BPA:lle altistuminen mitattiin alkuraskaudenaikaisesta virtsanäytteestä ja metylaatioprofiilit määritettiin napaverestä Illumina 450K tai EPIC sirulla. Äidit raportoivat lastensa käytösoireilua täyttämällä Child Behavior Checklist/1.5-5 (CBCL/1.5-5) kyselyn. Metylaatioon perustuva biomarkkeripistemäärä laskettiin LASSO-regression avulla. Yhteydet BPA altistuksen, biomarkkeripistemäärän ja käytöksen ongelmien välillä laskettiin lineaarisella sekä logistisella regressiolla.

Tulokset: Sekoittavien tekijöiden vakioimisen jälkeen, raskaudenaikainen BPA altistus lisäsi riskiä kliinisesti merkittävälle sisäänpäin ($p = .02$) ja ulospäin suuntautuneelle ($p = .04$) käytösoireilulle lapsuusiässä. Biomarkkeripistemäärä selitti 4.8% raskaudenaikaisen BPA altistuksen vaihtelusta ja lisäsi lähes tilastollisesti merkitsevästi kliinisesti merkittävän sisäänpäin ($p = .05$) ja ulospäin suuntautuneen ($p = .06$) käytösoireiluun riskiä lapsuusiässä.

Johtopäätökset: Raskaudenaikainen BPA altistus oli yhteydessä lapsuusiän käytösoireiluun. Myös metylaatioon perustuva BPA altistuksen biomarkkeri saattaa ennustaa lapsen käytösoireilua ja sitä tulisi tutkia lisää tulevissa tutkimuksissa.

Avainsanat – Nyckelord – Keywords

Bisfenoli A, BPA, käytösoireilu, DNA metylaatio

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Tiivistelmä – Referat – Abstract

Objectives: Bisphenol A (BPA) is commonly used plasticizer that has endocrine disrupting properties. Fetal exposure to BPA has been associated with offspring behavioural problems. These associations may be mediated through BPA-induced alterations in the offspring DNA methylation (DNAm). This study examined whether fetal BPA exposure associates with behavioural problems and whether DNAm biomarker score for early pregnancy BPA exposure is linked with behavioural problems in the offspring.

Methods: Participants were 442 mother-child pairs of the Finnish PREDO-cohort. I measured BPA from the early pregnancy urine samples and assayed DNAm in the cord blood with Illumina 450k or EPIC array. Mothers reported behavioural problems of their offspring with the Child Behaviour Checklist/1.5-5 (CBCL/1.5-5) at the mean child age of 3.8 years (SD = 1.0 years). I used LASSO regression to create a DNAm score for early pregnancy BPA exposure and tested the associations between BPA exposure, the DNAm score, and CBCL/1.5-5 scores with linear and logistic regressions.

Results: After adjustments, early pregnancy BPA exposure was associated with higher risk for clinically meaningful internalizing ($p = .02$) and externalizing ($p = .04$) behavioural problems in the offspring. The DNAm score included eight CpG sites, explained 4.8% of the BPA variation, and was borderline significantly associated with a risk for clinically meaningful internalizing ($p = .05$) and externalizing behavioural problems ($p = .06$).

Conclusions: Early pregnancy BPA exposure associated and DNAm biomarker for BPA exposure borderline associated with offspring behavioural problems. DNAm biomarker score for fetal BPA exposure showed promise and should be studied further in subsequent studies.

Avainsanat – Nyckelord – Keywords

bisphenol A, BPA, behavioural problems, DNA methylation

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Contents

1. Introduction

The estimated prevalence of child behavioural problems ranges from 7.9% (Sourander et al., 2001) to 11.1% (Larson et al., 1988). The behavioural problems are unpleasant for children and their relatives, but more importantly have wide variety of future implications. Childhood behavioural problems have been associated with shortened lifetime, higher risk for mental health problems and criminal behaviour (Scott, 2015). However, childhood behavioural problems are not deterministic and can be intervened. Early discovery of behavioural problems is a topic of high importance, since according to the Finnish current care guidelines (2018), the younger the child is when the interventions start, the more unlikely the suboptimal outcomes are. As for the interventions, the importance of identification of early risk factors has risen to recent literature.

One of the early risk factors for behavioural problems in childhood is early pregnancy Bisphenol A (BPA) exposure (Braun et al., 2011; Li et al., 2020). BPA is a commonly used plasticizer which is apparent in everyday life in modern society. It has endocrine disrupting properties and is especially harmful when exposed to in utero (Grohs et al., 2019; Balakrishnan, 2010). Measurement of fetal BPA exposure based on maternal sample is expensive. Thus, a biomarker based on easily available tissue such as, cord blood, could be preferred. As BPA has shown to alter DNA methylation (DNAm; Senyildiz et al., 2017), cord blood DNAm could both reflect BPA exposure during pregnancy and provide a biomarker for newborns risk for childhood behavioural problems. Using DNAm as an indicator of BPA exposure is challenging, since humans have approximately 20 million potentially methylated CpG sites (Antequera & Bird 1993). However, the individual DNAm alterations can be combined to a single biomarker score.

Finding a biomarker score of early pregnancy BPA exposure would provide a novel opportunity to reduce the prevalence of behavioural problems. The risk for behavioural problems could be detected after childbirth and the early environment could be modified to better suit the needs of the child. The parent directed interventions, which have been found to be most effective for preschool behavioural problems (Dretzke et al., 2009), could be started immediately after birth. Another benefit would be the possibility to advise parents of the heightened risk and thus guide them to seek help if there are indicators of behavioural problems in the future.

1.1. Child behavioural problems

The term child behavioural problem is defined by Finnish current care guidelines (2018) as behavioural problems which are more frequent or different by nature than is typical to the age group. In a sample of 374 Finnish children, the prevalence of behavioural problems, as measured by the Child Behaviour Checklist for ages 2-3 (CBCL/2-3) at the age of three years, was 7.4% (Sourander et al., 2001). The study also reported high prevalence of disobedience, defiance, difficulty in sitting still and a high activity level. The higher reported incidence of externalizing behavioural problems might be due to parents being biased towards reporting higher prevalence of externalizing than internalizing problems (de la Osa, 2016).

1.1.1. Implications of behavioural problem in childhood

Behavioural problems in childhood are known risk factors for significant physical, mental health and behavioural symptoms later in life. Childhood behavioural problems increase the likelihood of criminal activity and mood disorders in adulthood (Althoff, et al., 2014) and the risk for developing antisocial personality disorder (Blair, et al., 2014). In addition, childhood behavioural problems increase the risk for substance abuse, unemployment, problems in relationships in adulthood and are also associated with shorter lifetime (Scott, 2015).

The onset of behavioural problems as well as the duration of behavioural problems moderate the risk for physical, mental health and behavioural symptoms later in life (Odgers et al., 2008). The risks for problems in adulthood are higher in early onset behavioural problems. Also, the risk for problems in adulthood does not differ between children with behavioural problems only in childhood and children with no behavioural problems. In a Finnish longitudinal study consisting of 2556 males (Sourander et al., 2007), childhood behavioural problems increased the risk for psychiatric disorders or criminal activity in adulthood. The study reported that 4% of the sample had history with childhood behavioural problems and committed 26% of total criminal activity. In addition, 62% of the sample with history of childhood behavioural problems either manifested psychiatric disorder, got involved in criminal activity or both.

Etiology of behavioural problems include genetics and environment (Saudino et al., 2008). In addition to child's postnatal experiences and surroundings, environment also accounts for prenatal events and exposures (Jacobson, 1984). One of the recent interests in context of prenatal exposure is a common plasticizer called bisphenol A (Mustieles & Fernández, 2020).

1.2. Bisphenol A

Chemical formula for Bisphenol A (BPA) is 2,2-bis(4-hydroxyphenyl)propane (Wiley-VCH., 2016). BPA is used in manufacturing of epoxy resins and bisphenol A polycarbonate (BPA-PC; Allard, 2014). BPA-PC has a unique combination of key properties. It is extremely tough, transparent and has high heat distortion resistance. Due to BPA-PCs key properties, it has been utilized in water, milk and baby bottles, eyeglass lenses, sports equipment, medical and dental devices, line water pipes, canned food and register receipts to mention a few. As its wide range of applications suggests, it is the most economically important polycarbonate (Wiley-VCH., 2016).

The biological activity of BPA was discovered in 1938 (Dodds & Lawson, 1938) and in 1993 Krishnan et al., 1993 discovered that BPA can leach from BPA-PC when exposed to high pressure or heat. In the modern society, exposure to BPA is nearly impossible to avoid. According to Kang et al. (2006) humans expose to BPA at least by water, food, air and soil. Therefore, BPA has been consistently found in 95% of human urine (Calafat et al., 2005). The level of BPA exposure is affected by occupation and nutrition (Kang et al., 2006; Ribeiro et al., 2017).

In mammalian body, BPA can mediate estrogen receptor element (ERE)-dependent transcriptional activation or bind to androgen receptor thus preventing dihydrotestosterone from binding to the androgen receptor (Wade et al., 2003). Given the effects, BPA has been described as one on the most common endocrine-disrupting chemicals (Schug et al., 2011). In addition to androgen receptors, BPA exhibits antagonistic activity to the more active form of thyroid hormone (Allard, 2014; Geens, 2015).

The health concerns regarding bisphenol A have resulted in variety of actions by EU. Four evaluation were conducted between 2006 and 2011. The latest regulatory action by European Commission was in 2015 to limit the leach of BPA to the detection limit of 0.01 mg/kg in products aimed at children 3-years-old or younger. In other products aimed to be in contact with food the limit was lowered from 0.06 mg/kg to 0.05 gm/kg. In 2017, the Member State Committee of the European Chemicals Agency (ECHA) stated unanimously that bisphenol A is a substance of very high concern. The expansion of 0.01 mg/kg limit to cover pregnancy has gained support from recent studies since prenatal rather than postnatal exposure has been associated with behavioural outcomes (Grohs et al., 2019). Prenatal exposure to Bisphenol A has been suggested to be especially harmful,

since the brain goes through rapid development during pregnancy (Grohs et al., 2019) and is able to transfer through placenta (Balakrishnan, 2010).

The physical phenotypes correlating with prenatal BPA exposure range from obesity through prevalence of cancer to sperm epimutations. Prenatal BPA exposure has inverted the sex specific differences in the distance moved in open field tests and volume of locus coeruleus (Kubo et al., 2003). On psychopathological side, prenatal exposure to BPA has been linked with decreased dopaminergic neurons leading to the fetal origin of Parkinson's disease (Giulivo et al., 2016), decrease in asetylcholine neurotransmitter possibly resulting in memory impairments (Allard, 2014) and behaviour problems which are discussed in forthcoming sections.

1.2.1. Prenatal BPA exposure and offspring behaviour

In a recent meta study conducted by Mustieles and Fernández (2020), 10 out of 13 studies found associations between prenatal BPA exposure and increased offspring behavioural problems in childhood. Some of these studies found associations to internalizing behavioural problems or externalizing behavioural problems and some found associations to both behavioural problem categories. Of the three studies with no significant associations Miodovnik et al. (2011) studied the relationship between prenatal BPA exposure and autistic behaviour and Jensen et al. (2019) studied the relationship between prenatal BPA exposure and language development and attention-deficit and hyperactivity disorder (ADHD).

Interestingly, the association between prenatal BPA exposure on externalizing behavioural problems is stronger with exposure measured at week 16 of pregnancy compared to week 21 (Braun et al., 2009). The emphasis on early pregnancy BPA exposure is also supported by the three non-significant studies in the study of Mustieles and Fernández (2020) having measured urine BPA concentrations at either week 28 (Jensen et al., 2019), week 31 (Miodovnik et al., 2011) or at gestation (Kim et al., 2018).

The biological mediators of the association between prenatal BPA exposure and offspring behavioural problems are not well known. In previous studies white matter alterations (Grohs et al., 2019) and cord blood thyroid hormones (Li et al., 2020) have been suggested as potential biological mediators. In the study of Li et al., cord blood thyroid hormones were significantly associated with the behavioural problems but did not reach significance as mediator. White matter alterations

reached significance as mediators, but the study of Grohs et al. only studied mediating effect for internalizing behavioural problems since externalizing behavioural problems were not significantly predicted by prenatal BPA exposure. In addition, the behavioural problems were measured within six-month of the measurement of the white matter alterations, therefore making it harder to distinguish between cause and effect. Due to the alterations in DNAm associated with prenatal BPA exposure, DNAm could be another possible biological mediator.

1.3. DNA methylation

DNA methylation (DNAm) refers to a post replication modification where a methyl group is transferred from S-adenosyl-L-methionine to the DNA with the help of DNA methyltransferases (Tost, 2010). Although methylation can happen for multiple nucleotides, the most common variation is the methylation of 5 position of the pyrimidine ring of cytosine of the cytosine–guanine dinucleotide (CpG; Tost, 2010; Cavalli & Heard, 2019; Bender, 2004). Approximately 75 percent of CpG dinucleotides are methylated. The primary function of DNAm is to silence the transcription of the gene (Illingworth & Bird, 2009; Tost, 2010).

The CpG dinucleotides form clusters called CpG islands (CGI; Illingworth & Bird, 2009). Depending on the length restrictions assigned to CGIs, the number of these clusters in human genome has been approximated to be from 30 000 (Tost, 2010) to anywhere between 24 163 and 307 000 (Illingworth & Bird, 2009). Approximately 75 percent of transcriptional start sites and 88 percent of active promoters contain CGIs and therefore could be regulated by DNAm (Tost, 2010). The prevalence of CGIs in transcriptional and active promoter sites emphasizes the importance of DNAm in epigenetic modification.

Base pairs in DNA alternate between being methylated and non-methylated (Moore, Le & Fan, 2012). The fluctuation is influenced by the genotype, developmental mutations, environmental changes and neighbouring base pair methylations (Rakyan et al., 2011; Moore et al., 2012). Fluctuation is evident also within individual. Different tissues may have different base pairs methylated (Rakyan et al., 2004). In addition, methylation of different cells within a tissue, methylation of alleles within a cell and in rare cases methylation over DNA strands within an allele can vary (Rakyan et al., 2011).

Although the fluctuation of DNAm generates clear challenges, it also offers some new possibilities. The interplay between environment and methylation theoretically enables distinguishing individuals whose methylation has been affected by the change in environment. The recognition of these individuals would enable directing aid or environmental adjustments thus decreasing the risk for suboptimal outcomes. It for example provides a novel opportunity to study whether differences in prenatal environment can lead to different outcomes and whether epigenetics are mediating this effect. If clear differences are observed, the postnatal environment can be modified to provide the best surrounding for individual development.

1.3.1. Prenatal BPA exposure and DNA methylation

Higher levels of prenatal BPA exposure have been associated with global DNA hypomethylation and cell specific alterations in DNAm in human neuroblastoma cells (Senyildiz et al., 2017). In mice studies the prenatal BPA exposure bas been linked with hypomethylation primarily at hypermethylated loci (Yaoi et al., 2008). The hypomethylation is prevalent even with low dosage exposure during pregnancy of the mice.

The cord blood DNAm alterations associated with prenatal BPA exposure have been studied in epigenome-wide association studies (EWAS). In 2018, Junge et al. found two CpG sites significantly associated with BPA exposure measured at 34 weeks of gestation. The study comprised of 408 mother-child pairs. In 2019, Miura et al. studied the association between BPA exposure measured from cord blood and the cord blood methylation. The study comprised 277 mother-child pairs. Out of initial 426 413 CpG sites, 45 were identified as significant for the whole sample.

Interestingly the study of Miura et al. (2019) identified sex specific effects of exposure. 269 CpG sites were significantly associated with only male infants and 291 with only female infants. The methylation of DNA was also qualitatively different for different sexes. From all of the CpG sites associated with BPA, 98% (575 CpGs) were hypomethylated in female newborns. Out of the 269 CpG sites associated with only males, 88% were hypermethylated among male newborns.
The effect sizes of prenatal BPA exposure on individual CpG sites identified in EWAS are relatively low, thus precluding their use as an indicator of prenatal BPA exposure. In order to predict prenatal BPA exposure based on the newborn cord blood DNAm, one option is to construct polygenic methylation score based on the initial EWAS results.

1.4. Polygenic Methylation Score

Polygenic methylation score (PGMS) combines the effects of exposure to multiple CpG sites to a single score. Therefore, PGMS explains the effect of the exposure to a broader range of DNAm than associations to individual CpG sites. Methylated CpG sites are called methylation variable positions (MVPs; Barros & Offenbacher, 2009). Since there are around 19 000 genes (Ezkurdia et al., 2014) and approximately 20 million potentially methylated CpG sites (Antequera & Bird 1993), the sample size typically limits the number of MVPs used to explain the exposure. Therefore, instead of using the whole methylation profile, MVPs with known or suspected associations to the exposure are commonly chosen as initial MVPs. EWAS can be used to acquire initial knowledge on individual associations between MVPs and exposure.

After initial MVPs have been identified, the covariation of MVPs has to be taken into account. One possibility is to select only MVPs with low correlation. After accounting for covariation, the remaining MVPs are used to produce a weighted linear combination (de Vlaming & Groenen, 2015). The linear combination can be formed with multiple different regression methods and the method should be selected given the number of observations and MVPs.

As the number of MVP's typically exceeds the number of observations, variations of least absolute shrinkage and selection operator (LASSO) and ridge regression are often chosen over variations of ordinary least squares regression. De Vlaming and Groenen (2015) argue that variations of ridge regression should be selected over LASSO if the number of MVPs contributing to the phenotype is assumed to be relatively large which is typical to psychological phenotypes. The argument bases on tendency of LASSO regressions to perform additional MVP pruning, since the overfitting is penalized by a parameter measured by the sum of absolute regression weights (Ranstam & Cook, 2018). In ridge regression all chosen MVPs are included into the model and overfitting is penalized by a parameter measured by the sum of squared regression weights (Hoerl & Kennard, 2000). The selection of LASSO over ridge regression is typically preferred when the end result is desired to include as few estimators as possible. To balance between the two methods, elastic net regression uses weighted proportions of both LASSO and ridge regression penalizing factors.

DNAm data can be enriched with other features of the individual. For example, the Rett syndrome is almost exclusive prevalent in girls (Smeets et al., 2012) and therefore including sex to the PGMS

could result in more accurate predictions. If the model is conducted with LASSO or ridge regression, the enriching features can be excluded from the effect of parameter penalizing overfitting (de Vlaming & Groenen, 2015). This would ensure that the effect of enriching parameters do not dilute as the effect of individual MVPs dilute.

The most studied PGMS is the estimator of DNAm age, epigenetic clock (Horvath & Raj, 2018), but PGMS have been utilized in estimating for example DNAm gestation age (Knight et al., 2016) prenatal stress (Provençal et al., 2019; Suarez et al., 2020) and antenatal depression (Suarez et al., 2018). The use of cord blood PGMS as an indicator of prenatal BPA exposure has some advantages: Instead of indicating the level of exposure, cord blood DNAm indicates effects of accumulating exposure to a biological phenotype. Therefore, accounting for individual thresholds in exposure tolerance. Finally, cord blood is easily accessible tissue in every pregnancy.

1.5. Research questions

1. Does maternal early pregnancy BPA level predict behavioural problems in the offspring?
2. Does cord blood polygenic methylation score for early pregnancy BPA exposure in the cord blood predict behavioural problems in the offspring?

2. Methods

2.1. Study population

The Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction (PREDO) Study comprises of 1,083 pregnant women and their singleton children born from 2006 to 2010 in Finland. The recruitment took place in consecutive order when these women attended their first ultrasound screening at 12 to 13 weeks of gestation in hospital maternity clinics of the study. Of the recruited women, 973 had one or more risk factors for preeclampsia and intrauterine growth restriction (IUGR), and 110 had no known risk factors.

In total, 443 women provided urine samples at the 12 + 0–13 + 6 gestational weeks + days of gestation. BPA was able to be assayed from 442 women. Of this sample, 221 mothers reported child behavioural problems with the Child Behavior Checklist for ages 1.5-5 (CBCL/1.5-5) questionnaire at the mean age of 3.8 (SD =1.0) years. Cord blood DNAm was available for 347 participants with

the data of BPA exposure and 186 mother-child dyads had DNAm measured from cord blood and CBCL/1.5-5 questionnaire data available for child behavioural problems. Sample characteristics are described in Table 1.

The Ethics Committees of the Helsinki and Uusimaa Hospital District and the participating hospitals approved the study protocol. Written informed consent was obtained from all participating women.

2.2. Measurement of Bisphenol A

Bisphenol A (BPA) was analysed from the spot urine samples donated at 12 + 0–13 + 6 weeks + days of gestation. Samples were analysed by the Finnish Institute for Health and Welfare, according to a validated, and accredited standard (SFS-EN ISO/IEC 17025:2017) by FINAS Finnish Accreditation Service, testing laboratory T077) procedures. For quantitation at the beginning of the analytical procedure, isotope labelled internal standards for BPA were added to the 0.25 ml urine sample. To determine the total amount of BPA (conjugated as well as non-conjugated), the conjugated species were hydrolyzed by use of β-glucuronidase/sulfatase (Helix pomatia H2; glucuronidase activity 304199 units/ml , sulfatase activity 2976 units/ml Sigma Aldrich Laboratories, Inc., St. Louis, MO).

Prior to enzymatic hydrolysis sample volume was adjusted to 0.50 ml with water consisting 0.5 µl of glucuronidase/sulfatase solution. The enzyme reaction (deconjugation) took place in 37 °C for 20 h. After the hydrolysis sample was centrifuged with Eppendorf 5810 (Hamburg, Germany) at 2600 g for 10 min. The BPA was separated and quantified with Thermo Scientific UltiMate 3000 Rapid Separation LC system (Germering, Germany) connected to Thermo Finnigan TSQ Quantum Discovery MAX triple quadrupole mass spectrometer (Waltham, MA, USA). Details of the LC-MS/MS parameters are described elsewhere (Porras et al., 2020; Rajasärkkä et al., 2014). All the analyte concentrations were blank corrected and the limit of detection (LOD) was 0.32 µg/L. Concentrations below the LOD were given a value of LOD/√2– for statistical analyses (Hornung & Reed, 1990). The BPA concentrations were transformed with natural logarithm to normalize the BPA distribution.

Table 1

Sample characteristics

	Entire Sample With Urine Bishenol A Data (N = 442)	**Sample With Urine Bisphenol A and CBCL/1.5-5 Questionnaire data (N = 221)**		**Sample With Urine Bisphenol A and Fetal Cord Blood DNA Methylation Data (N = 347)**		**Sample With Fetal Cord Blood DNA Methylation and CBCL/1.5-5 Questionnaire Data (N = 186)**	
	Mean (SD) or n (%)	**Mean (SD) or n (%)**	**p**	**Mean (SD) or n (%)**	**p**	**Mean (SD) or n (%)**	**p**
Maternal Characteristics							
Education							
Primary Education	13 (3%)	8 (4%)	.70	12 (3%)	.72	7 (4%)	.65
Secondary Education	194 (44%)	93 (42%)	.42	149 (43%)	.58	78 (42%)	.42
Lower Tertiary Education	93 (21%)	47 (21%)	.88	74 (22%)	.95	38 (20%)	.71
Upper Tertiary Education	127 (29%)	73 (33%)	.39	108 (31%)	.60	63 (34%)	.31
Depression at CBCL measurement[a]							
Normal	173 (39%)	168 (77%)	.97	138 (75%)	.73	138 (75%)	.73
Mild Disturbance	28 (6%)	27 (12%)	.99	24 (13%)	.84	24 (13%)	.84
Borderline	14 (3%)	14 (6%)	.93	13 (7%)	.72	13 (7%)	.72
Moderate or Higher	10 (2%)	9 (4%)	.87	8 (4%)	.97	8 (4%)	.97
Smoking							
No	407 (92%)	207 (94%)	.96	328 (95%)	.58	176 (95%)	.61
Yes	28 (6%)	14 (6%)	.96	19 (5%)	.58	10 (5%)	.61
Child Characteristics							
Sex							
Boy	232 (52%)	116 (53%)	.68	183 (54%)	.83	97 (52%)	.60
Girl	194 (44%)	104 (47%)	.68	158 (46%)	.83	89 (48%)	.60
Age at CBCL/1.5-5 measurement (months)[b]	46.8 (13.1)	45.5 (12.1)	.28	44.7 (11.5)	.08	44.3 (11.1)	.04*

Notes: p Refers to p-value comparing given sample to the entire sample with urine bisphenol A data.
a: sample size in entire BPA sample = 225, sample size in BPA + DNA methylation sample = 183
b: sample size in entire BPA sample = 237 sample size in BPA + DNA methylation sample = 192
*** p < .05**

2.3. DNA Methylation

Fetal cord blood samples were collected according to standard procedures. DNA was extracted at the National Institute for Health and Welfare, Helsinki, Finland, and the Institute for Molecular Medicine Finland, University of Helsinki, Finland. Methylation analyses were performed at the Max Planck Institute of Psychiatry in Munich, Germany. DNA was bisulphite converted using the EZ-96 DNAm kit (Zymo Research, Irvine, CA). In 876 (74%) samples, genome-wide methylation status of more than 485,000 CpG sites was measured using the Infinium Human Methylation 450k BeadChip (Illumina Inc., San Diego, CA) and 301 (26%) samples with the Infinium Human Methylation 850k BeadChip (Illumina Inc., San Diego, CA) according to the manufacturer's protocols. The arrays were scanned using the iScan System (Illumina Inc., San Diego, CA). The quality control pipeline was set up using the R-package minfi (Aryee, 2014). Samples with maternal blood contamination (n = 9) were excluded according to a method described elsewhere (Morin et al., 2017).

Methylation beta-values were normalized using the funnorm function. Any probes on chromosome X or Y, probes containing single-nucleotide polymorhpisms (SNP)s and cross-hybridizing probes were excluded. Furthermore, any Cgs with a detection p-value > 0.01 in at least 50% of the samples were excluded. Finally, ComBat was used to check and adjust for the batch effects (slide and well).

To control for the potential effects of cell type heterogeneity in fetal umbilical cord blood, cord blood cell counts at birth were estimated for 7 cell types (nucleated red blood cells, granulocytes, monocytes, natural killer cells, B cells, $CD4^+$ T cells, and $CD8^+$ T cells) using the method of Salas et al. (2018) which is also incorporated in the R-package minfi (Aryee, 2014).

2.4. Child Behavioural problems

When the children were on average 3.8 (SD = 1.0) years, mothers filled-in CBCL/1.5-5 (Achenbach & Rescorla, 2000) to report the behavioural problems of their child. The CBCL/1.5-5 questionnaire consists of seven syndrome scales and five DSM-Oriented scales. Syndrome scales are aggressive behaviour, attention problems, sleep problems, withdrawn, somatic complaints, anxious/Depressed and emotionally reactive. DSM-oriented scales on the other hand are oppositional defiant problems, attention deficit/hyperactivity problems, pervasive developmental problems, anxiety problems and affective problems.

I grouped the syndrome scales into two higher order factors: internalizing behavioural problems and externalizing behavioural problems (Gjone, 1997). Internalizing behavioural problems consist of anxious/depressed, withdrawn and somatic complaints. Externalizing behavioural problems is derived from aggressive behaviour and attention problems. I also calculated the total problems scale based on all the syndrome scales. As suggested in the CBCL/1.5-5 manual, I used a cut-off at 60 t-transformed points to indicate clinically meaningful behavioural problem for the internalizing, externalizing and total behavioural problem scales (Achenbach & Rescorla, 2000). This score has been suggested as the cut-off between no behavioural problems and borderline behavioural problems (Rescorla, 2005). In the sample of this study, 13 (6%), 12 (5%), and 10 (5%) of the children belonged to the group with clinically meaningful internalizing, externalizing and total behavioural problems, respectively. The behavioural problem t-transformed points are described in Supplementary Table S1.

In the literature, the mean test-retest correlation for CBCL/1.5-5 is approximately 0.85, with range between 0.8 and 0.9 (Rescorla, 2005). The validity of the CBCL/1.5-5 has been tested against DSM diagnoses (de la Osa, 2016) and other child behaviour questionnaires such as Caregiver–Teacher Report Form (C–TRF; Rescorla, 2005). The CBCL/1.5-5 also has shown good psychometric properties across multiple cultures (Kristensen, 2010).

2.5. Covariates

I first studied the crude associations between early pregnancy BPA exposure and child behavioural problems with no covariates at the mean age of 3.8 (SD = 1.0) years. In the fully adjusted model, I included factors that could potentially influence this association (Malanchini et al., 2019; Park, 2018; Spann, 2016; Liu, 2011): child's sex, child's age at follow-up (months), maternal education, maternal smoking during pregnancy, and maternal self-reported depressive symptoms at follow up.

To study the associations between PGMS for early pregnancy BPA exposure and child behavioural problems at 3.8 years, in the crude model, I used two genomic principal components to control for population stratification, cell type estimates to control for variation in the cell type proportions in the cord blood samples, and the methylation array (450K or EPIC) as covariates. 450K methylation array was used in 309 (89%) and EPIC methylation array in 38 (11%) measurements. In the fully adjusted model, I further included child's sex, child's age at follow-up (months), maternal

education, maternal smoking during pregnancy, and maternal self-reported depressive symptoms at follow up. N=8 mothers were dropped in the analyses due to missing data in the covariates.

Data on maternal education and smoking were extracted from the birth register. Maternal education was further categorized to primary education, secondary education, lower tertiary education, and upper tertiary education. I categorized smoking during pregnancy to two classes: those who did not smoke during pregnancy and those who smoked for at least some time during pregnancy.

Mothers self-reported depressive symptoms with Beck Depression Inventory - Second Edition (BDI-II; Beck et al., 1996) at the same follow-up as they filled-in the CBCL/1.5-5. I categorized BDI-II to four classes: minimal (BDI $\leq$ 13), mild (14 $\leq$ BDI < 20), moderate (20 $\leq$ BDI < 28) and severe (BDI > 29). Due to only one participant belonging to severe depression group, I merged severe and moderate to a single group named moderate or higher (BDI $\geq$ 20).

2.6. Statistical methods

I used logistic regression to first test the associations of early pregnancy BPA exposure and PGMS for early pregnancy BPA exposure with the risk for clinically meaningful behavioural problems in the offspring. I also used linear regression to test the associations of early pregnancy BPA exposure and PGMS for early pregnancy BPA exposure with the behavioural problems in the offspring. Moreover, I tested if early pregnancy BPA exposure and PGMS for early pregnancy BPA exposure associated differently with offspring behavioural problems in boys and girls by including cross-product terms (sex x BPA exposure or sex x PGMS for early pregnancy BPA exposure) in the models. For behavioural problems, CBCL/1.5-5 higher order factors, syndrome scales and DSM-oriented scales were evaluated. All calculations have been conducted with R statistical software, version 4.0.0 (R Core Team, 2020).

2.7. DNA Methylation biomarker score

To calculate cord blood PGMS for early pregnancy BPA exposure, I first selected initial pool of CpG sites that showed association with prenatal BPA exposure in the EWAS by Miura et al. (2019). The study consisted of 277 mother-child pairs and they found 45 CpG sites that showed significant association ($p < 0.0001$) with exposure to BPA.

I used LASSO regression to select a combination of CpG sites that showed the lowest Mean Squared Error (MSE) in N=347 mothers with cord blood methylation and early pregnancy BPA data available. I used 10-fold cross validation to reduce the possibility of overfit. The resulting minimum lambda after fitting the elastic net regression was approximately 0.05. Altogether 8 CpG sites were selected to form the DNAm biomarker score based on MSE. The pseudo R2 for the score was 4.8%. The mean score was 0.41 (SD = 0.09) and median score was 0.41. The CpG sites and their corresponding coefficients are described in Table 2.

Table 2
CpG sites selected to biomarker score and their coefficients

CpG site	**Coefficient**
cg11532800	-0.03
cg05714773	-0.06
cg14048686	-0.01
cg11820931	-0.05
cg20800606	0.03
cg16789995	0.02
cg20666585	0.03
cg27624753	< 0.01

3. Results

Table 1 shows the characteristics of the analytic samples: Those with BPA and CBCL/1.5-5 data (n = 221) did not differ from the entire sample with BPA data (p-values > .28). In addition, those with BPA and cord blood DNAm data (n = 347) did not differ from the entire sample with BPA data (p-values > .08). In those with cord blood DNAm and CBCL/1.5-5 data, the child age at CBCL/1.5-5 measurement was on average 2.5 months younger than in those with maternal BPA data (p-value = .04).

3.1. Association between early pregnancy BPA exposure and offspring behavioural problems

After adjustments of child's sex, child's age at follow-up (months), maternal education, maternal smoking during pregnancy and maternal self-reported depressive symptoms at follow up, higher

early pregnancy BPA levels predicted higher risk for clinically meaningful CBCL/1.5-5 externalizing behavioural problems (OR = 2.18, 95% CI 1.05 – 4.6, p = .04) and higher risk for clinically meaningful CBCL/1.5-5 internalizing behavioural problems (OR = 2.25, 95% CI 1.15 – 4.55, p = .02), but did not predict risk for clinically meaningful CBCL/1.5-5 total behavioural problems (OR = 1.85, 95% CI 0.8 – 4.31, p = .18) at the mean child age of 3.8 years (Table 3). These associations were not significant in the crude models (p-values > .12; Tables 3). There were also no significant associations in the models considering CBCL/1.5-5 scores as continuous variables (p-values > .45; Supplementary Table S2).

In the fully adjusted models, higher early pregnancy BPA exposure also predicted higher anxious/depressed (B = 0.43, 95% CI 0.08-0.77, p = .02) and emotionally reactive (B = 0.56, 95% CI 0.03-1.08, p = .04) syndrome scale scores and higher anxiety disorder (B = 0.63, 95% CI 0.06-1.2, p = .03) DSM-oriented score. In the crude models, the association for anxious/depressed syndrome scale scores were significant (B = 0.38, 95% CI 0.03-0.73, p = .03), but emotionally reactive (B = 0.40, 95% CI -0.15-0.96, p = .15) and anxiety disorder (B = 0.54, 95% CI -0.04-1.12, p = .07) syndrome scale scores turned non-significant. Other associations between early pregnancy BPA exposure and CBCL/1.5-5 syndrome scales or DSM-oriented scales were not significant (p-values > .14).

Table 3 also shows that including BPA to fully adjusted model increased pseudo proportions of variance explained by 5 percentage points for clinically meaningful externalizing behavioural problems, 5 percentage points for clinically meaningful internalizing behavioural problems and 2 percentage points for clinically meaningful total behavioural problems when compared to model with only covariates. Furthermore, interactions between child sex and bisphenol A were not significant in either models for any of the behaviour problems (p-values > .19).

Table 3
Associations of early pregnancy BPA exposure with mother-reported clinically meaningful behavioural problems at the age of 3.8 years.

		Bisphenol A				
			95% CI			
Behavioural problems	**Odds Ratio**	**Lower CI**	**Upper CI**	**p**	**n**	**ΔR^2**
Crude[a]						
Total	1.25	0.66	2.24	.46	221	-
Internalizing	1.51	0.89	2.51	.12	221	-
Externalizing	1.38	0.78	2.35	.24	221	-
Fully adjusted[b]						
Total	1.85	0.8	4.31	.18	213	.02
Internalizing	2.25*	1.15	4.55	.02	213	.05
Externalizing	2.18*	1.05	4.60	.04	213	.05

***** p < .001. ** p < .01. * p < .05; 95% CI = 95% confidence interval**
a = Unadjusted model
b = Model adjusted for child's sex, child's age at follow-up, mothers' education, mothers' smoking during pregnancy, and maternal self-reported depressive symptoms at follow up

3.2. Association between PGMS for early pregnancy BPA exposure and offspring behavioural problems

After adjustments of child's sex, child's age at follow-up (months), maternal education, maternal smoking during pregnancy and maternal self-reported depressive symptoms at follow up, higher scores in the PGMS for early pregnancy BPA exposure did not predict higher risk for clinically meaningful CBCL/1.5-5 internalizing behavioural problems (OR = 2.42, 95% CI 1.01 – 6.31, p = .05), clinically meaningful CBCL/1.5-5 externalizing behavioural problems (OR = 2.77, 95% CI 1.02 – 9.35, p = .06) or clinically meaningful CBCL/1.5-5 total behavioural problems (OR=2.77, 95% CI 0.86 – 12.87, p = .12) at the mean child age of 3.7 years (Table 4). These associations were not significant in the crude models (p-values > .21; Table 4). There were also no significant associations in the models considering CBCL/1.5-5 scores as continuous variables (p-values > .29; Supplementary Table S3).

After adjustments, higher scores in the PGMS for early pregnancy BPA exposure were associated with higher attention problem syndrome scale score (B = 0.53, 95% CI 0-1.05, p = .05) and higher ADHD DSM-oriented scale score (B = 0.58, 95% CI -0.01-1.18, p = .05) scores. Without adjustments neither of these associations were significant (attention problems: B = 0.44, 95% CI -0.08-0.97, p = .10 and ADHD: B = 0.49, 95% CI -0.10-1.08, p = .10). Other associations between PGMS for early pregnancy BPA exposure and CBCL/1.5-5 syndrome scales or DSM-oriented scales were not significant (p-values > .17).

Table 4 also shows that including PGMS for early pregnancy BPA exposure to crude model increased pseudo proportion of variance explained by 6 percentage points for externalizing clinically meaningful behavioural problems, 4 percentage points for clinically meaningful internalizing behavioural problems and 4 percentage points for clinically meaningful total behavioural problems when compared to model with only covariates. Including PGMS for early pregnancy BPA exposure increased the pseudo proportion of variance explained by 28 percentage points for clinically meaningful externalizing behavioural problems, 30 percentage points for clinically meaningful internalizing behavioural problems and 19 percentage point for clinically meaningful total behavioural problems when compared to model with only covariates. Interactions between child sex and PGMS for early pregnancy BPA exposure were not significant in either models for any of the behaviour problems (p-values > .49).

Table 4
Associations of PGMS for early pregnancy BPA exposure to mother-reported clinically meaningful behavioural problems at the age of 3.7 years.

	PGMS for early pregnancy BPA exposure					
		95% CI				
Behavioural problems	**Odds Ratio**	**Lower CI**	**Upper CI**	**p**	**n**	**ΔR^2**
Crude[a]						
Total	1.18	0.56	2.46	.44	161	.04
Internalizing	1.31	0.68	2.53	.42	161	.04
Externalizing	1.54	0.78	3.1	.21	161	.06
Fully Adjusted[b]						
Total	2.77	0.86	12.87	.12	158	.28
Internalizing	2.42	1.01	6.31	.05	158	.30
Externalizing	2.77	1.02	9.35	.06	158	.19

***** p < .001. ** p < .01. * p < .05; 95% CI = 95% confidence interval**
a = Model adjusted for two genomic principal components, cell type estimates and methylation array
b = Model adjusted for two genomic principal components, cell type estimates, methylation array, child's sex, child's age at follow-up, mothers' education, mothers' smoking during pregnancy, and maternal self-reported depressive symptoms at follow up

4. Discussion

In this study BPA exposure in early pregnancy was significantly associated with clinically meaningful offspring internalizing and externalizing behavioural problems at the mean age of 3.8 years after adjusting for child's sex, child's age at follow-up, maternal education, maternal smoking during pregnancy, and maternal self-reported depressive symptoms at follow up.

These finding are in line with earlier results from multiple different cohorts. In USA, Braun et al. (2009) found significant positive associations between maternal early pregnancy BPA exposure and externalizing behavioural problems of girls at the age of two. In a Canadian cohort, maternal urinary BPA levels at an average of 12.1 weeks of gestation were significantly associated with internalizing behavioural problems in boys at the average age of 3.4 years (Braun et al., 2017) and

urinary BPA levels at second trimester were associated with internalizing behavioural problems at the age of four (Grohs et al., 2019).

Of studies linking early pregnancy BPA exposure with childhood behavioural problems to my knowledge only Li et al. (2020) have previously found significant associations to both internalizing and externalizing behavioural problems. Interestingly, similar to the methodology of this study, the children were categorized to two groups based on behavioural problems. The discrepancy between results with continuous and dichotomous behavioural problem definitions might be due to suggested nonlinearity of relationship between endocrine-disrupting chemicals with estrogenic activity and outcomes (Wade et al., 2003). The nonlinearity has been taken into account in most studies by logarithmically transforming BPA concentrations, but as the shape of the association between prenatal BPA exposure and behavioural problems is not known for certain, dichotomous outcome measures might provide needed simplification for the assumptions of the shape of the association.

In addition to clinically meaningful internalizing and externalizing behavioural problems, maternal BPA levels in early pregnancy associated linearly with scores on the anxious/depressed and emotional reactivity syndrome scales and anxiety disorder DSM-oriented scale after adjusting for confounders. These findings are in line with previous studies. In a study conducted by Braun et al. (2017), early pregnancy BPA exposure was associated with emotional reactivity. In addition, both Braun et al. (2011) and Li et al. (2020) have found significant associations between early pregnancy BPA exposure and emotional reactivity and anxiety.

In order to study whether cord blood DNAm could be used as a biomarker between maternal BPA exposure in early pregnancy and behavioural problems, I calculated PGMS for early pregnancy BPA exposure. The resulting PGMS consists of eight weighted CpG sites. These sites were derived from an EWAS that examined the association of prenatal BPA exposure on cord blood DNAm (Miura et al. 2019). The associations from the PGMS to clinically meaningful offspring internalizing and externalizing behavioural problems at the age of 3.8 years after adjusting for confounders were borderline clinically significant.

In previous studies, brain structure alterations of the child at the mean age of 3.7 (Grohs et al., 2019) and cord blood thyroid hormones (Li et al., 2020) have been suggested as possible biomarkers for the association between early pregnancy BPA exposure and offspring behavioural

problems. Of brain structure alterations, mean diffusivity in the splenium was found to be a significant mediator of the association between prenatal BPA exposure and externalizing behavioural problems. Of cord blood thyroidal hormones, thyroid stimulating hormone was significantly associated with prenatal BPA exposure and internalizing behavioural problems but did not mediate the relationship. Previously found potential biomarkers for the association between prenatal BPA exposure and behavioural problems do not contradict the findings of this study, since biomarkers are not mutually exclusive. On the contrary, finding multiple biomarkers rather strengthens the assumption of prenatal BPA exposure affecting offspring behaviour through biology.

The strength of the association between cord blood DNAm and behavioural problems could have been dampened by the ethnic difference between the sample used in this study compared to the sample of base EWAS used by Miura et al. (2019). The sample used in the study of Miura et al. was recruited from Toho Hospital, located in Sapporo, Japan and the sample of this study was of European origin. The methylation of DNA is known to differ significantly between cultures (Galanter, 2017). Therefore, even though the association between early pregnancy BPA and behavioural problems has gained support from multiple different ethnicities (Mustieles & Fernández, 2020) some methylation sites affected by BPA exposure in the sample of this study might have been excluded from the construction of DNAm biomarker score.

Besides the ethnicity of the sample, the base EWAS of Miura et al. (2019) differed from the sample used in this study by the methodology of BPA measurement, which might have affected the results. DNAm typically differs between tissues (Rakyan et al., 2004) and the effect of prenatal BPA exposure can be different during different stages of pregnancy (Braun et al., 2009). The BPA exposure was measured from cord blood at gestation in the study of Miura et al. and from early pregnancy urine sample in this study.

The strength of the association might also have been dampened by the previously suggested nonlinearity of the relationship between BPA and outcomes (Wade et al., 2003). The DNAm biomarker score as such is constructed by adding weighted estimated methylation levels of DNA sites to a single score. Applying the weights require predetermined assumptions of the relationship. If the predetermined assumption does not match the true underlying relationship, the score might not be as accurate as possible. To account for the possibility of nonlinear relationship, I logarithmically transformed the level of early pregnancy BPA exposure as is common in studies

examining the relationship between early pregnancy BPA exposure and behavioural problems (Braun et al., 2011; Braun et al., 2009; Braun et al., 2017; Casas et al., 2015).

The main limitation of this study is the relatively small number of the participants with clinically meaningful behavioural problems. The second limitation is the recruitment strategy, with enrichment of women with risk factors for pre-eclampsia and Intrauterine Growth Restriction (IUGR). The recruiting strategy may restrict the generalization of the results of this study. However, it is highly unlikely that my results showing association between maternal BPA levels in early pregnancy and behavioural problems in early childhood are due to Type 1 error or may not generalize to mothers without risk factors for pre-eclampsia or IUGR, as the association is in line with several earlier studies with a similar design. Also, the typical association between prenatal BPA exposure and behavioural problem subcategories mainly consists of anxiety and emotional reactivity, which are in line with the findings of this study. The third limitation is measurement of BPA concentrations from a single spot urine sample.

The single spot urine sampling is a limitation because the level of exposure is assumed to reflect a broader exposure during the pregnancy and BPA is known to have relatively short half-life, which means that traces of BPA are detectable in urine for only a short period of time (Lee & Jacobson, 2015). Due to the short half-life, the intraclass correlation coefficient (ICC) of one spot urine sample for ideal BPA sample is approximately 0.38. A posterior disattenuation with study specific ICC can increase the ICC to 0.89 (Vernet et al., 2019). Recent literature encourages repeated biospecimen collections (Perrier et al., 2016), although the single spot urine sampling has support in reflecting widespread exposure in the population with reasonable accuracy (Ye et al., 2011). In addition, if any bias would occur because of the urine sampling, the single spot sample-based misclassification of BPA exposure would bias the results towards null.

The main strength of the study is the relatively large sample size. In earlier studies examining associations between prenatal BPA exposure and offspring behavioural problems, sample sizes have ranged from 98 (Grohs et al., 2019) to 812 (Braun et al., 2017) and majority having sample size under 300 (Mustieles & Fernández, 2020). The sample is also ethnically homogenous and well characterized and the proportion of BPA concentrations under the limit of detection is only 1.4%.

In summary, BPA exposure in early pregnancy was significantly associated with clinically meaningful offspring internalizing and externalizing behavioural problems at the mean age of 3.8.

Although the associations between DNAm biomarker score and clinically meaningful offspring behavioural problems were not statistically significant, the strength of associations gives support for using DNAm as a biomarker for the relationship between early pregnancy BPA exposure and clinically meaningful offspring internalizing and externalizing behavioural problems. These results provide further evidence of the harmfulness of early pregnancy BPA exposure to the fetus and indicate possibility of the effect of early pregnancy BPA exposure being mediated by cord blood methylation. Since the results on DNAm biomarker score were not conclusive, the biomarker should be developed further in subsequent studies.

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Supplements

1. Supplementary Table S1

Supplementary Table S1

Behavioural problem T-score descriptive statistics

	Sample With Urine Bisphenol A and CBCL/1.5-5 Questionnaire data (N = 221)	Sample With Fetal Cord Blood DNA Methylation and CBCL/1.5-5 Questionnaire Data (N = 186)
	Mean (SD)	Mean (SD)
Behavioural problem T-scores		
Total	45.1 (9.1)	45.2 (9.1)
Internalizing	44.9 (9.3)	44.7 (9.5)
Externalizing	46.4 (8.8)	46.6 (8.8)
Behavioural problem syndrome scale T-scores		
Aggressive Behaviour	52.4 (4.8)	52.5 (5.0)
Attention Problems	51.7 (2.9)	51.8 (3.1)
Sleep Problems	53.8 (5.0)	53.7 (5.0)
Withdrawn	52.6 (4.4)	52.7 (4.6)
Somatic Complaints	53.8 (5.9)	53.6 (5.9)
Anxious/Depressed	51.0 (2.6)	51.1 (2.8)
Emotionally Reactive	52.4 (4.1)	52.3 (4.1)
Behavioural problem DSM-oriented T-scores		
Oppositional Defiant Problems	52.8 (4.3)	52.9 (4.4)
ADHD	51.8 (3.3)	51.9 (3.5)
Pervasive Developmental Problems	53.0 (5.2)	53.1 (5.4)
Anxiety Disorder	52.3 (4.3)	52.3 (4.5)
Affective Disorder	52.8 (4.2)	52.8 (4.3)

2. Supplementary Table S2

Supplementary Table S2

Associations of early pregnancy BPA exposure with mother-reported behavioural problems at the age of 3.8 years.

		Linear Models Bisphenol A				
			CI			
Behavioural problems		**Coefficient**	**Lower CI**	**Upper CI**	**p**	**n**
Crude[a]						
	Total	-0.07	-1.17	1.07	.91	221
	Internalizing	0.09	-1.32	1.17	.89	221
	Externalizing	-0.39	-1.19	1.37	.52	221
	Aggressive Behaviour	0.03	-0.61	0.68	.92	221
	Attention Problems	-0.04	-0.43	0.36	.86	221
	Sleep Problems	0.22	-0.45	0.89	.52	221
	Withdrawn	0.09	-0.51	0.69	.77	221
	Somatic Complaints	-0.27	-1.06	0.52	.51	221
	Anxious/Depressed*	0.38	0.03	0.73	.03	221
	Emotionally Reactive	0.40	-0.15	0.96	.15	221
	Oppositional Defiant Problems	0.03	-0.55	0.61	.93	221
	ADHD	0.12	-0.32	0.57	.59	221
	Pervasive Developmental Problems	0.16	-0.55	0.86	.66	221
	Anxiety Disorder	0.54	-0.04	1.12	.07	221
	Affective Disorder	0.07	-0.50	0.64	.80	221
Fully adjusted[b]						
	Total	0.37	-0.75	1.49	.51	213
	Internalizing	0.47	-0.75	1.68	.45	213
	Externalizing	-0.05	-1.17	1.07	.93	213
	Aggressive Behaviour	0.24	-0.38	0.85	.44	213
	Attention Problems	0.06	-0.32	0.45	.75	213
	Sleep Problems	0.47	-0.16	1.09	.14	213
	Withdrawn	0.16	-0.44	0.75	.61	213
	Somatic Complaints	-0.15	-0.92	0.63	.71	213
	Anxious/Depressed*	0.43	0.08	0.77	.02	213
	Emotionally Reactive*	0.56	0.03	1.08	.04	213
	Oppositional Defiant Problems	0.19	-0.37	0.74	.51	213
	ADHD	0.22	-0.21	0.66	.31	213
	Pervasive Developmental Problems	0.24	-0.45	0.93	.50	213
	Anxiety Disorder*	0.63	0.06	1.20	.03	213
	Affective Disorder	0.22	-0.31	0.75	.42	213

***** p < .001. ** p < .01. * p < .05**
a = Unadjusted model
b = Model adjusted for child's sex, child's age at follow-up, mothers' education, mothers' smoking during pregnancy, and maternal self-reported depressive symptoms at follow up

3. Supplementary Table S3

Supplementary Table S3

Associations of PGMS for early pregnancy BPA exposure to mother-reported behavioural problems at the age of 3.7 years.

		Linear Models PGMS for early pregnancy BPA exposure				
			CI			
Behavioural problems		**Coefficient**	**Lower CI**	**Upper CI**	**p**	**n**
Crude[a]						
	Total	0.20	-1.29	1.69	.79	161
	Internalizing	0.49	-1.08	2.05	.54	161
	Externalizing	-0.02	-1.46	1.41	.97	161
	Aggressive Behaviour	0.38	-0.45	1.21	.37	161
	Attention Problems	0.44	-0.08	0.97	.10	161
	Sleep Problems	0.03	-0.82	0.88	.95	161
	Withdrawn	0.11	-0.65	0.88	.77	161
	Somatic Complaints	0.12	-0.87	1.11	.81	161
	Anxious/Depressed	0.08	-0.40	0.55	.75	161
	Emotionally Reactive	-0.03	-0.69	0.62	.92	161
	Oppositional Defiant Problems	-0.02	-0.77	0.73	.96	161
	ADHD	0.49	-0.10	1.08	.10	161
	Pervasive Developmental Problems	0.36	-0.56	1.28	.44	161
	Anxiety Disorder	0.05	-0.70	0.80	.89	161
	Affective Disorder	0.09	-0.63	0.81	.80	161
Fully adjusted[b]						
	Total	0.38	-1.04	1.80	.60	158
	Internalizing	0.83	-0.71	2.36	.29	158
	Externalizing	0.03	-1.36	1.43	.96	158
	Aggressive Behaviour	0.56	-0.25	1.38	.17	158
	Attention Problems*	0.53	0.00	1.05	.05	158
	Sleep Problems	0.18	-0.63	1.00	.66	158
	Withdrawn	0.28	-0.51	1.06	.49	158
	Somatic Complaints	0.36	-0.63	1.34	.48	158
	Anxious/Depressed	0.16	-0.33	0.64	.53	158
	Emotionally Reactive	0.07	-0.60	0.73	.85	158
	Oppositional Defiant Problems	0.07	-0.66	0.80	.85	158
	ADHD*	0.58	-0.01	1.18	.05	158
	Pervasive Developmental Problems	0.56	-0.38	1.49	.24	158
	Anxiety Disorder	0.22	-0.54	0.98	.57	158
	Affective Disorder	0.25	-0.44	0.95	.47	158

***** p < .001. ** p < .01. * p < .05**

a = Model adjusted for two genomic principal components, cell type estimates and methylation array

b = Model adjusted for two genomic principal components, cell type estimates, methylation array, child's sex, child's age at follow-up, mothers' education, mothers' smoking during pregnancy, and maternal self-reported depressive symptoms at follow up